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14. ABSTRACT
The central hypothesis of this project is that the function of the cerebral vasculature is impaired in response to primary blast loading. A shock tube is being utilized to study overpressure alteration of cerebral blood flow and disruption of the blood-brain barrier in the living rat, along with change of passive and active response of isolated rat cerebral vessels. Key accomplishments during the first year of this award include construction of a shock tube, development of a computational model to simulate blast production using a shock tube, and modification of a vessel testing system to accommodate rat cerebral vessels and to allow fluid circulation with temperature and pH control.

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Blast brain injury; cerebrovascular injury and dysfunction; shock tube

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Introduction

Recent increased exposure of American troops to blast loading has revealed an unexpectedly high incidence of symptoms consistent with traumatic brain injury (TBI). The mechanisms of such overpressure injuries of the brain are not understood. The cerebral blood vessels play a key role in maintaining homeostasis of the central nervous system (CNS), and non-blast models of TBI have demonstrated impairment of the cerebrovascular system, but the response of the vasculature to blast loading has not been defined. The central hypothesis of this project is that the function of the cerebral vasculature is impaired in response to primary blast loading. Two aims are underway to test this hypothesis. Aim 1 will characterize the level of overpressure required to produce alteration of cerebral blood flow (CBF) and disruption of the blood-brain barrier (BBB) in the living rat. Aim 2 will determine blast loading levels required to produce alteration of passive and active response in isolated rat cerebral vessels. These aims together will more clearly define the influence of blast loading on the cerebral vasculature and will begin to determine how impairment of vascular function may contribute to blast brain injury. Findings from this research will help to focus the design of more effective methods of prevention and treatment of CNS injuries due to overpressure.

Body

Each of the tasks listed in the approved Statement of Work (revised 3/17/09) is presented as a section heading below, followed by a description of progress within that task.

Task: Construct shock tube

This task has been completed. The shock tube is now operational and is currently undergoing initial tests to characterize response as a function of independent variables.

Through collaboration with Prof. Daniel Kirk at the Florida Institute of Technology (FIT), we have constructed a shock tube that is approximately 4 feet long and 1 inch in diameter; this is a second-generation design of a shock tube in use at the FIT facility. We originally planned to construct a much larger tube and to place the target inside the tube. The primary advantage of the smaller tube, however, is the ability to isolate the target from forces due to venting and to thus be able to study mechanisms of primary blast independent of secondary, tertiary, and other effects.

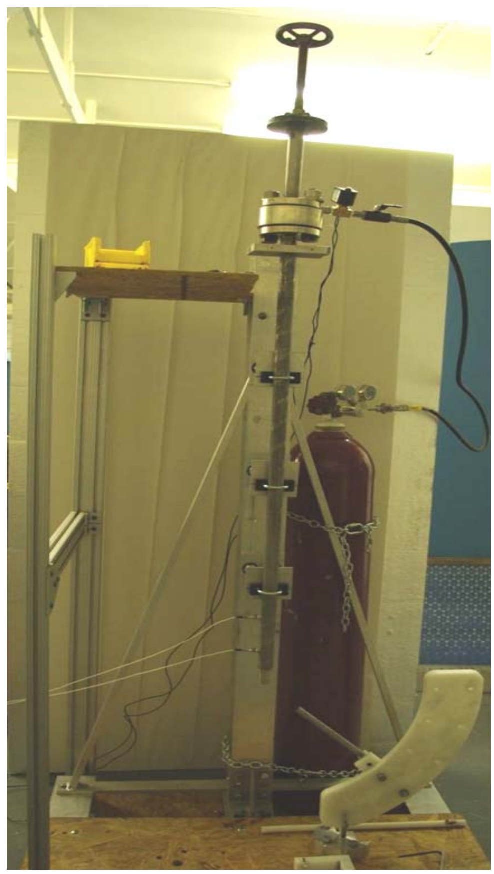


Figure 1 Shock tube

An image of the device is shown in Figure 1. It is mounted vertically, with the driver section at the top, on an aluminum I-beam frame that also supports a platform at its base. Driver length can be adjusted between 1 and 4 inches while the driven length is fixed at approximately 50 inches. Adjustment of driver section length is one independent variable controlling the resulting wave characteristics. During operation, the two sections are separated by a thin steel membrane that deforms under increasing driver pressure (provided by a compressed Nitrogen tank) until it contacts cutting blades fixed in the tube and ruptures, allowing the high driver pressure to propagate into the driven section of the tube and form a shock wave. As the wave exits the end of the tube at the bottom, it expands axisymmetrically. The characteristics of the expanding wave vary as a function of both the distance *r* from the end of the tube and the angle θ from its axis (Fig. 2). Desired loading conditions are thus obtained by positioning the target at a particular location in the field. The end of the driven section is machined to a sharp edge to minimize reflections off the end of the tube.

The platform at the base of the device provides attachment for fixtures supporting pressure transducers (PCB model 102B15 high frequency ICP pressure sensor) that can be oriented to measure either static or reflected pressure. Two pressure sensors are available for measurement in the field, and two are mounted perpendicular and flush to the inner surface of the driven section to allow measurement of static pressure within the tube; these latter sensors are positioned 3.9 inches (10 cm) along the axis of the tube from one another to allow calculation of wave speed based on the difference in pressure rise times at the two locations. Pressure data are collected using a high speed (2.5 Ms/s/ch) multifunction data acquisition card (NI PXI-6133; National Instruments) controlled by LabVIEW software (National Instruments). Specimen deformation will also be monitored using a Phantom Miro eX4 high speed camera (Vision Research) that is currently on order.

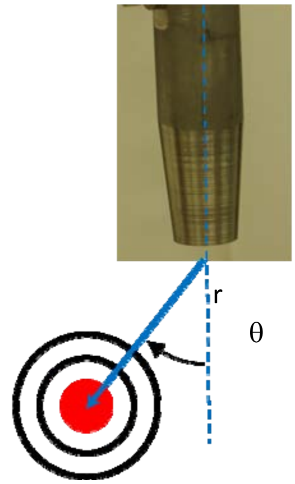
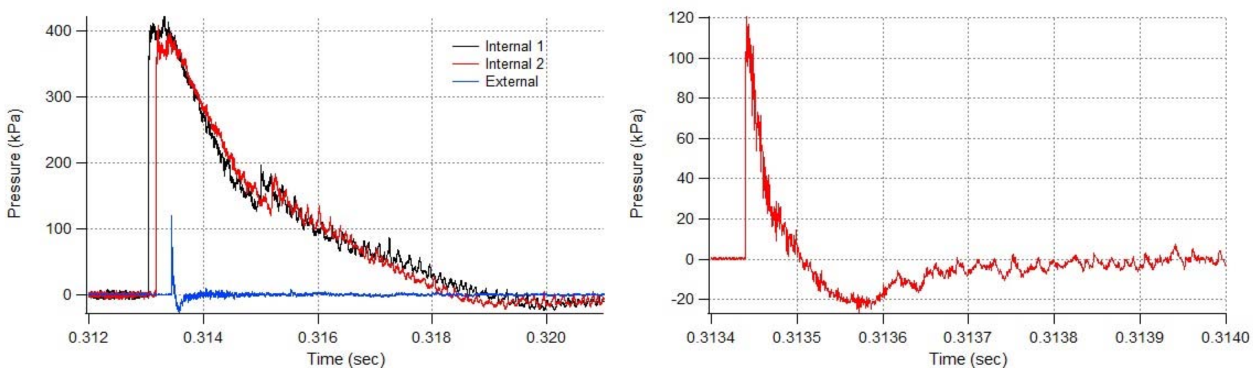


Figure 2 Radius and angle defining target position in pressure field at exit of tube

The shock tube system has only recently reached the operational stage, so it has not yet been used with experimental targets. Pressure data have, however, been collected in initial firings of the tube and are shown in Figure 3. Data corresponding to both the interior tube locations and a sensor located at approximately 60 deg and 3 inches in the target field, oriented to give reflected pressure, are presented. Driver pressure was 4830 kPa (700 psi), and driver length was 4 inches. As shown, the pressure wave at the target shows the desired characteristics of a Friedlander curve, with an immediate rise in pressure followed by a positive impulse and a subsequent negative impulse, all occurring over approximately 0.5 milliseconds.



(a) **(b)**

Figure 3 Pressure-time histories of (a) both internal sensors and one external sensor (reflected orientation) and (b) a magnified plot of the external sensor only. External sensor location was at 3 in and 60 deg.

Task: Construct fixtures for modification of existing isolated vessel ex vivo testing apparatus
Fixtures have been modified to accommodate rat middle cerebral arteries, which are significantly smaller than the human cerebral vessels the fixtures were originally designed for.

The testing system has also been upgraded to allow fluid circulation and control of both temperature and pH. The only subtask remaining is to construct a fixture to position the vessel during blast testing; this is expected to be complete by February 2010.

Task: Animal committee approval
This task is complete. Approval was originally obtained for experiments on mice, but a change to rats was made to allow study of a larger, more accessible system.

Task: Shock tube experiments on rats
To begin February 2010. Qualitative control data for blood-brain barrier disruption have been collected, as shown in Fig 4. Animals were perfused with FITC Dextran and then euthanized. Figure 4 shows Dextran (green) overlaid with immunoglobulin (red), clearly marking the interior wall of intact blood vessels of the rat brain. In collaboration with Prof. Patrick Tresco of the Keck Center for Tissue Engineering here at the University of Utah, brain slices and isolated vessels will also be stained to explore any damage to the internal elastic lamina of the vessels (Fig. 5).

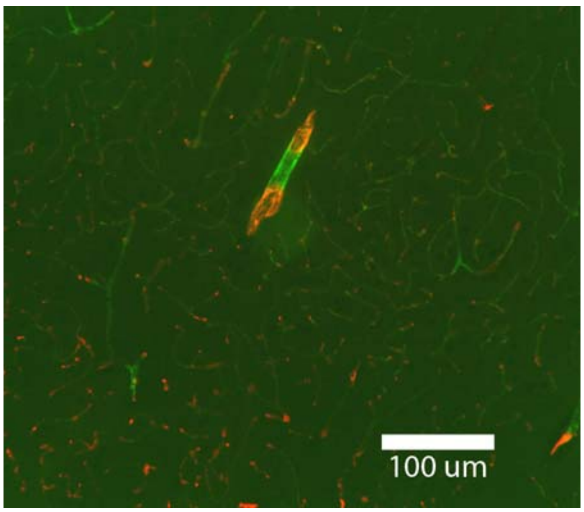


Figure 4 FITC Dextran (green) and immunoglobulin (red) in control rat brain

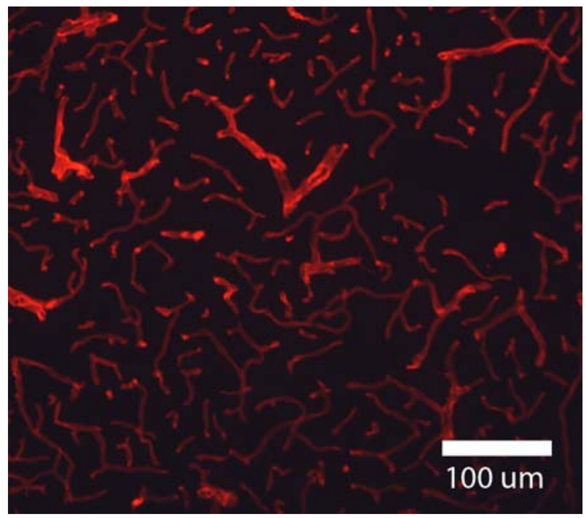


Figure 5 Laminin in control rat brain

Graduate students are also currently receiving training from an experienced animal technician for performance of cerebral blood flow measurements via microsphere deposition.

Task: Shock tube experiments on isolated blood vessels from rats

Blast experiments on isolated vessels are expected to begin February 2010, but procedures for resecting the rat middle cerebral artery (MCA) and attaching it to the testing device have been developed. Preliminary tests on mechanical properties have also been conducted.

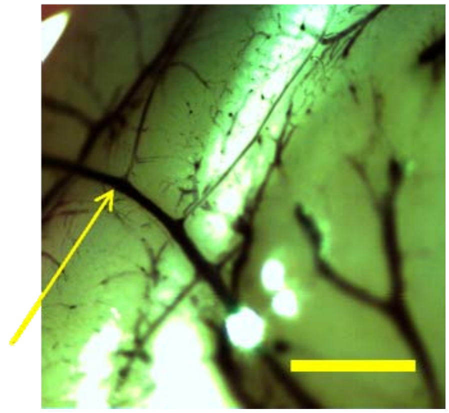


Figure 6 Stained middle cerebral artery (arrow) and branches *in situ* at base of resected rat brain; scale bar is 1 mm

Prior to brain removal, rats are perfused with Nigrosin dye. Once the brain is removed from the skull, the dye provides contrast to aid in visualization of vascular structure and allow careful resection of the MCA with its branches (Fig 6). A cross-section is then cut from the isolated vessel, its branches are tied off, and it is attached to 35 or 36 gauge hypodermic stainless steel needles at each end and connected to the testing device (Fig. 7). Once attached to the device, the vessel can be tested under various combinations of pressure and axial stretch while monitored by a video camera attached to the computer.

Because of the small scale involved, the process of resecting the rat MCA from the brain takes a significant amount of time, so there is some concern that the vessel may degrade and lose the healthy active response we seek to study. To alleviate this risk, the resection will be conducted in cold saline and the tissue will be regularly rinsed. In addition, full initial tests of vessel response both before and after blast will be conducted with either carotid or femoral arteries obtained from rats. The resection-to-testing time of these vessels is significantly shorter and will allow both development of the full procedure and proof-of-concept testing with little concern of tissue degradation.

Figure 7 Resected rat MCA attached to testing device (not shown); scale bar is 0.5 mm

Task: Computational modeling of isolated blood vessels

This task is in progress. Computational modeling of the shock tube at the University of Utah has been added to this task, and fluid-structure simulations of the blast wave – soft tissue interactions will follow. A smaller-scale fluid-structure model specifically studying blood vessel blast loading will be developed by a collaborator at UC Berkeley, Prof. Tarek Zohdi.

In order to better understand how changing driver pressure, driver length, and target position influence the blast wave at the target, we've developed a numerical model of the shock tube in collaboration with Prof. Todd Harman of C-SAFE (Center for the Simulation of Accidental Fires and Explosions) at the University of Utah. The model is simulated using the Implicit Compressible Eulerian (ICE) CFD algorithm to solve the Navier-Stokes equations. Use of this model not only allows the development of intuition for complex nonlinear shock wave phenomena, but it is also being used to predict blast wave characteristics at various candidate target locations as a function of driver pressure and length. Because wave characteristics will change significantly over the target field in our experimental setup, this simulation tool will provide vital data for guiding target placement. Figure 8 shows the static pressure field downstream from the end of the tube at a single time during one such simulation. This CFD algorithm has previously been coupled with the Material Point Method to simulate fluid-structure interactions during explosions and fires by C-SAFE. We anticipate that this tool will be extended to further simulate blast-tissue interactions measured during our experiments.

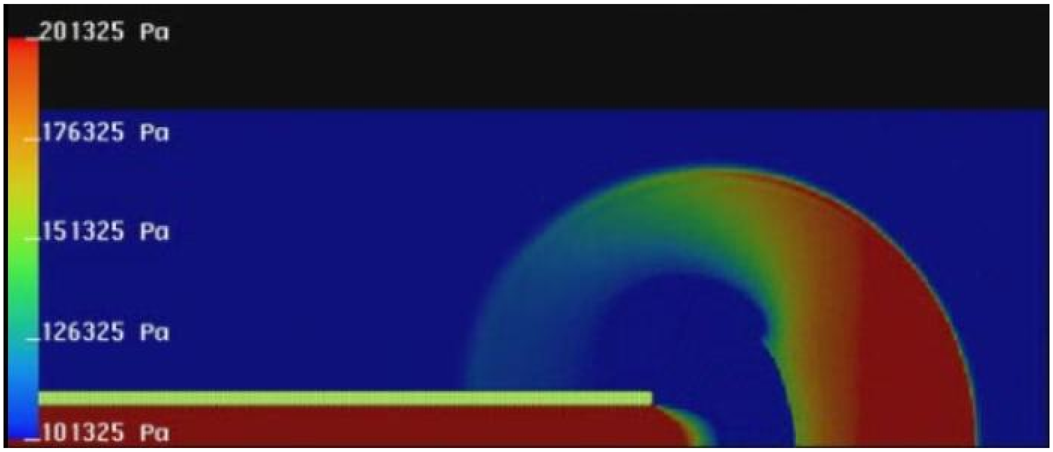


Figure 8 Pressure distribution at one point in time following exit of blast wave from the end of the shock tube (note that all negative pressures are displayed as being equivalent to atmospheric with the chosen pressure scale of this image)

An additional computational model simulating blast wave-blood vessel interaction is also under development by Prof. Tarek Zohdi at UC Berkeley as a part of this project.

Key research accomplishments

Key accomplishments emanating from this research are limited at this point since experiments are just getting underway, but major milestones accomplished to-date include the following:

- Construction of a shock tube to simulate free-field blast overpressure
- Development of a computational model to simulate axisymmetric blast wave characteristics after exit from a 1 inch diameter shock tube
- Modification of the isolated vessel testing system to accommodate a rat MCA and to allow fluid circulation with temperature and pH control

Reportable outcomes

- Poster and presentation detailing project progress at Military Health Research Forum 2009: Monson, K. L., Bell, E. D., Kunjir, R., Bokil, M. M., Harman, T. B., Yeoh, A. S., Manley, G. T. Cerebrovascular injury in blast loading. Military Health Research Forum, Aug 31-Sept 3, 2008, Kansas City, MO. Poster is included in the appendix.

Conclusions

Significant progress has been made toward accomplishing the specified aims, and the project is on schedule for completion by the end of 2010. Findings will more clearly define the influence of blast loading on the cerebral vasculature and will begin to determine how impairment of vascular function may contribute to blast brain injury. Findings from this research will help to focus the design of more effective methods of prevention and treatment of CNS injuries due to overpressure.

References

N/A

Appendices

- Poster detailing project progress at Military Health Research Forum 2009: Monson, K. L., Bell, E. D., Kunjir, R., Bokil, M. M., Harman, T. B., Yeoh, A. S., Manley, G. T. Cerebrovascular injury in blast loading. Military Health Research Forum, Aug 31-Sept 3, 2008, Kansas City, MO.

P28-3

Cerebrovascular Injury in Blast Loading

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HYPOTHESIS / AIMS

The central hypothesis of this project is that the function of the cerebral vasculature is impaired in response to primary blast loading.

AIM 1: Characterize the level of overpressure required to produce alteration of cerebral blood flow (CBF) and disruption of the blood-brain barrier (BBB) in the living rat.

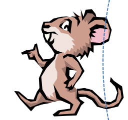
AIM 2: Determine blast loading levels required to produce alteration of passive and active response in isolated rat cerebral vessels.

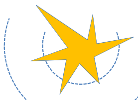
Impact: Findings from this research will clarify the response of the cerebrovasculature to blast and help focus efforts in both blast injury treatment and prevention.

BACKGROUND

- Blast injury has traditionally been associated with damage to air-containing organs, such as the lungs, eardrums, and intestines.
- Recent exposure of American troops to blast loading has revealed an unexpectedly high incidence of symptoms consistent with traumatic brain injury (TBI) (Cernak, I. *et al*, J Trauma 1999). Animal models have confirmed neurological damage resulting from blast (Cernak, I. *et al*, J Trauma 2001; Risling, M. *et al*, Swedish Defense Research Agency 2002).
- Mechanisms of TBI resulting from primary blast are not understood, including whether the associated brain injury is a result of primary trauma or if other trauma-induced pathophysiology produces secondary brain injury.
- The cerebral blood vessels play a key role in maintaining homeostasis of the central nervous system. Cerebrovascular injury / dysfunction is a key part of the blast injury signature.
- Non-blast models of TBI have demonstrated impairment of the cerebrovascular system (Langfitt, T. W. *et al*, Ann Surg 1977; Thomale, U. W. *et al*, J Neurotrauma 2002; Golding, E. M. *et al*, J Cereb Blood Flow Metab 2003; Eucker, S. *et al*, J Neurotrauma 2005; Maikos, J. T. and Shreiber, D. I., J Neurotrauma 2007), but it is not clear if this disruption of function is due to injury of the blood vessels themselves or to damage of surrounding tissues and changes in the biochemical environment.
- The response of the vasculature to blast loading has not been defined (DeWitt, J Neurotrauma 2009).

METHODOLOGY





Aim 1

- Rats will be exposed to a blast pulse generated by a compressed air-driven shock tube.
- Animals will be secured outside the tube and off-axis to eliminate secondary and tertiary blast effects.
- Animals will be positioned to receive the blast either frontally or laterally, testing a sub-hypothesis that impulse direction is an important factor.
- Three overpressure levels will be utilized to generate dose-response data.
- Fluorescent microsphere deposition methods will be used to measure CBF both before and at 30 min, 4, 24, and 48 hours after blast exposure.
- BBB disruption will be characterized at the same time points.

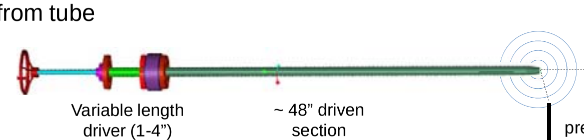
Aim 2

- Rat middle cerebral arteries (MCA) will be loaded into an isolated vessel testing system and subjected to combinations of axial stretch and pressure around physiological conditions to define their baseline passive mechanical properties; vessels will also be exposed to various neurotransmitters to characterize their active response characteristics.
- Vessels will then be subjected to blast load levels matching those used in Aim 1; any blast-induced active response will be recorded.
- Vessels will be subjected to the same set of mechanical and biochemical tests to define any changes.

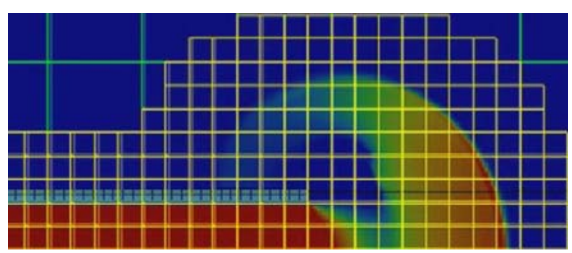
PROGRESS

Shock Tube Development / Construction

- Develop shock in 1” diameter tube with animal placed off-axis to eliminate contributions of blast wind, following design of Kirk *et al* at Florida Institute of Technology (Kirk, D. R. et al, 38th Fluid Dynamics Conf, AIAA, 2008)
- Characteristics of blast varied by changing distance / angle from tube



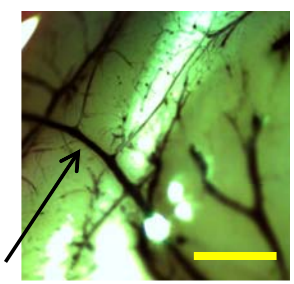
- Computer simulation helps characterize complex pressure field associated with diffracted wave at tube exit



Pressure field in 2-D axisymmetric simulation 2.4 ms after diaphragm rupture; simulation conducted using MPMICE – Implicit, Continuous fluid, Eulerian approach for compressible fluids; Material Point Method for rigid tube; MPMICE operates within the Uintah Computational Framework

Isolation / Testing of Rat Middle Cerebral Artery

- Perfuse vessels with dye prior to/just after euthanasia to better visualize vessel and branches
- Dissect vessel off brain, tie off branches, and secure to custom 36 gauge hypodermic needles using suture and cyanoacrylate



Perfused and dyed rat MCA and branches *in situ* (scale bar = 1 mm)

Rat MCA isolated between needles in testing system (scale bar = 0.5 mm)

Other Notable Areas of Progress

- Circulation loop in vessel testing setup to incorporate flow of temperature and pH-controlled media, providing a more realistic mechanical and biochemical environment

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